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# ON THE TRANSMISSION OF IMMUNITY FROM MOTHER TO OFFSPRING. A STUDY UPON SERUM HEMOLYSINS IN GOATS.*†

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## I. INTRODUCTION.

The inheritance, by the progeny, of newly acquired biological qualities from either or both parents has been a question of long standing. It has attracted the attention of numerous investigators, particularly physicians, since the beginning of scientific medicine and biology. Certain tendencies to disease were considered to be transmissible from parents to offspring (inherited). On the other hand, observations were recorded of cases where the mother, shortly after having recovered from a disease, gave birth to a child, and the child upon exposure to the same disease showed resistance against the infection. As an example of this character may be mentioned variola (Lereboullet, Buchner).[1] Burckhardt[2] made similar observations upon infants born from mothers vaccinated (vaccinia) during late pregnancy. In small-pox an intrauterine infection of the fetus may occur, in which case not a true inherited, but an actively acquired, resistance results in the child; possibly the same thing occurs in vaccinia. The transient resistance which infants commonly show against many infections during the first weeks of life, while not fully understood, is probably for the most part of maternal origin.

As is evident, the problem has been beset with difficulties, and very little progress was made toward a solution until the newer discoveries in immunity became known. Immunity actively acquired against specific infectious agents or certain toxins offered itself as a means, readily applicable, by which an insight might be gained concerning the general laws of inheritance, and at the same time afford practical data on the subject of immunity. Following the discovery of the exciting causes of many of the infectious diseases, and the isolation of these specific organisms, animal experimentation was undertaken by several investigators. Chauveau,[3] in 1880, found that by vaccinating with anthrax organisms the slightly susceptible Algerian sheep, during gestation, their offspring were born immune against this organism. These results were supported later by other workers using symptomatic anthrax (Arloing, Cornevin, and Thomas,[4] Kitasato[5]).

A means had now been acquired by which fairly definite tests could be employed under conditions subject to control. More exact methods were introduced for actively immunizing animals against various antigens. The resulting degree of immunity became subject to standardization, thus establishing a firmer basis for studying the problem. Subsequently much important work appeared which threw more light upon the question.

The publication, in 1892, of the results of Ehrlich's[6] basic studies marked a new epoch in the development of the subject. He carried out immunization experiments by feeding mice the toxalbumins, abrin, ricin, and robin. It was found that the young from immune mothers acquired an immunity, which he considered as passive, that is, a passage over of the specific antibodies to the fetus before birth, and then through the milk to the nursling after birth. He proved by placing normal young upon immune nurses, that the milk transmitted specific antibodies which were absorbed by the nurslings, and caused their immunization. Also, he found that highly immunized males, when crossed with normal females, failed to endow the young with resistant powers against the specific toxin, and so concluded that the idioplasm of the sperm did not carry over immunity to offspring.

In opposition to Ehrlich's assertion that the immunized male alone was unable to transmit an acquired immunity to his descendants, several workers brought forward experiments which conflicted with his results. Among these workers may be mentioned Gley and Charrin,[7] Tizzoni and Cattani,[8] and Tizzoni and Centanni.[9] Ehrlich and Hübener[10] (1894) repeated similar experiments, using tetanus toxin against both guinea-pigs and mice, with results which sustained Ehrlich's first findings. In a critical review of the opposing work, they pointed out certain experimental conditions in each case which they considered invalidated the results. The investigations of Wernicke[11] (1895), Vaillard[12] (1896), and others (Remlinger,[13, 65] Dieudonné,[14] Bulloch,[15] etc.) fully supported Ehrlich's findings. So at the present time the view is, for the most part, generally accepted that acquired immunity is not inherited by the offspring in the ontogenetic sense. In the mammalia apparently the germ plasm of neither parent plays an appreciable rôle in transmitting an acquired immunity to young.*

In discussing the question of the transmission of an acquired immunity from parents to the offspring, attention must be directed to the mother. The specific immunity of the offspring is a passive condition, and not a biological inherited condition of long standing. Therefore the subject may be transferred from the province of biological heredity (germ plasm) to the field of practical medicine and prophylaxis, and be considered from that standpoint.

## II. GENERAL REVIEW OF WORK UPON THE SUBJECT OF TRANSMISSION OF IMMUNITY FROM MOTHER TO OFFSPRING.

An extensive literature pertaining to the transmission of immunity from mother to offspring has developed in the past two decades. Since I have had occasion to make a rather exhausted review of this literature, perhaps it may be of interest if a brief outline of the more important papers dealing with the subject be submitted. Special consideration will be given those papers which are important either from a historical standpoint, or those of fundamental value in the development of the subject. No attempt will be made to abstract even briefly the numerous articles, but instead a provisional classification of antibodies and antigens will be outlined, and under these headings the references will be catalogued. These papers, in general, concern the transmission of specific antibodies to the young by actively immunized animals. In some instances, simply the presence of antibodies in the milk of such actively immunized animals are reported. Purposely the work upon passive immunization of the mother animal is omitted, since in our studies attention is directed only to actively immunized mothers and their offspring.

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* It may be of interest to mention in this connection the work of Klemperer (*Arch. f. exper. Path. u. Pharmacol.*, 1893, 31, p. 356), who showed that the yolk of eggs from hens which had been actively immunized against tetanus showed antitoxin. Dzierzgowski (*Centralbl. f. allg. Path. u. path. Anat.*, Ref., 1901, 12, p. 715) confirmed these results with hens actively immunized against diphtheria. He states that chicks hatched from such eggs showed diphtheria antitoxin in their blood serum.

## A. ANTIBODIES OF WELL DIFFERENTIATED TYPES.

1. *Antitoxins.*—Animal experimentation has been carried out upon the following subgroups of toxins: *a*) Plant toxalbumins. As mentioned earlier, animals immunized against abrin, ricin, and robin transmitted the antibodies to their own young, or to normal sucklings (Ehrlich[6]).

*b*) Bacterial, extracellular toxins. (1) Tetanus. Actively immunized rats and rabbits (Tizzoni and Cattani[16]), guinea-pigs and mice (Ehrlich and Hübener[10]), and mare (Ransom[17]) transmitted an immunity to their offspring. It is of historical interest that Brieger and Ehrlich,[18] in a study upon antitoxin-bearing milk from goat immunized against tetanus, published the first immunity curves. (2) Diphtheria. In diphtheria, clinical cases have been reported in which mothers recovering from the disease have apparently transmitted antitoxins to their infants (Kayser,[19] and others). But it has been shown that a high percentage of infants from normal mothers show antitoxic properties in their sera after birth; therefore the interpretation of the above observations are open to question (Fischl and v. Wunschheim[20]). Among animal observations, guinea-pigs (Wernicke,[11] Anderson[21]) and mare (Salomonsen and Madsen[22]) have been shown to transmit immunity or antibodies against diphtheria to their young. (3) Pyocyaneus. In some cases rabbits (Charrin and Gley[23]), when immunized during the course of pregnancy, transmitted, to a certain degree, specific protective powers to their young. (4) Symptomatic anthrax. As mentioned, different workers have found that a certain degree of immunity is transmitted from the recently immunized mother to her young (Arloing, Cornevin and Thomas,[4] Kitasato[5]). (5) Vibrio Nasik (vibriolysin). Wegelius[24] reports that goats and rabbits, when immunized either shortly before conception or during gestation, transmit the specific antibodies to their young.

*c*) Animal venoms. Fraser[25] states that a cat which was immunized against snake venom during the course of pregnancy gave birth to young which suckled mother; one kitten, when given two minimum lethal doses (subcutaneously) on the 57th day, showed marked resistance against the venom with only very slight symptoms, from which it soon recovered; the other kitten on the 69th day was given three fatal doses of venom from which it failed to survive.

*d*) Ferments. Morgenroth[26] immunized goats against rennin and found in a study of the milk that the specific antibody was present in considerable amounts. No example was found in the literature where studies were made upon the transmissibility of antibodies of this type from mother to young.

2. *Agglutinins.*—Including *a*) bacterial, and *b*) hemagglutinins. *a*) Bacterial agglutinins. (1) Typhoid. In view of the fact that much work has been done upon this group of agglutinins both (*a*) clinically (patients) and (*b*) experimentally (animals), a brief outline of the two subgroups will be given. (*a*) Among the clinical observations of possible intrauterine transmission of typhoid agglutinins from mother to fetus, a number of cases have been reported in which the mother aborted during the course of the disease. The body fluids from fetuses ranging in age from three to six months failed to show specific agglutinins (Etienne,[27] Charrier and Apert,[28] Dogliotti[29]). However, Scholtz[30] reported a strong agglutinating reaction from fluids of a seven-months' fetus. In other cases (Schumacher,[31] Stäubli,[32] Mosse and Daunic[33]) blood, taken from the fetal end of cord at birth (about or full term) of children born from mothers who had suffered during the course of pregnancy from typhoid, gave positive reactions. Other cases are reported (Castaigne,[34] Courmont and Cade[35]) in which infants, taking the milk from mothers who were infected as late as two months (one case) after having given birth to young, also showed positive agglutinating sera. However, this was not always the case, since in a few instances (Kasel and Mann[36]) the milks (2) were positive (1:25)—the blood agglutinated 1:50—but no reaction was given by the suckling's sera. The mothers in this series were infected 2–21 years before parturition. In another instance (Achard and Bensaude[37]) a mother contracted the disease while nursing the child (age ?); the milk showed a low agglutinating value (1:10); the blood of the nursling was negative. In the other cases the milk from the mother when tested showed a positive reaction, with few exceptions. (*b*) The published studies upon animal experimentation add nothing further to the results already cited. Widal and Sicard[38] reported positive results in young rabbits born from a mother which was inoculated with typhoid bacillus six days before parturition. The heart blood taken from young at time of birth, however, was much weaker in agglutinating power than the mother's blood. Guinea-pigs actively immunized during pregnancy in most cases transmitted agglutinins to their young (Remlinger,[13] Jurewitsch,[39] Stäubli[40]). It is noteworthy that a goat which was highly immunized late in pregnancy gave birth to a kid whose blood showed no specific agglutinins (Schumacher[31]). This kid had not suckled before a blood sample was taken.

Among other organisms whose specific agglutinins have been reported as passing to the young from the actively immunized mothers, the following may be mentioned: (2) *B. tuberculosis;** specific agglutinins passed from infected mother to child (Lagriffoul and Pages[41]). (3) Cholera; specific agglutinins were transmitted from guinea-pigs immunized during pregnancy to the fetus or the young (Achard,[42] Dieudonné[14]). (4) Proteus; specific agglutinins were found in the serum of young guinea-pigs from immunized mothers (Achard[42]).

Specific agglutinins for the above and other microorganisms have been demonstrated in the milk of immunized animals. But, as is evident, it does not follow that agglutinins are transmitted with any regularity from mother, either intrauterine or through the milk, to the offspring. For instance, a goat showing a reaction of 1:400 against anthrax failed to pass much agglutinin over to the still-born young; the kids' sera reacted in 1:10 dilution, which was much less than was normally present in the mother's blood before inoculation (Gengou[43]). (*b*) Hemagglutinins. Kraus[44] reported that transmission of immune hemagglutinins did not take place in a kid born from a mother which had been immunized against red blood cells of sheep, but these immune bodies were demonstrated in the goat's milk. In infant's blood serum (cord) at time of birth, natural hemagglutinins were found to be less in amount than in the mother's serum (Halban,[45] Halban and Landsteiner[46]), although there was much variation, depending upon the source of the test corpuscles.

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* Figari (*Centralbl. f. Bakt.*, I, Ref., 1907, 39, p. 75), reports that hens fed upon tubercle bacillus preparations after a time showed specific agglutinins in their eggs.

3. *Precipitins.*—Specific precipitins against human blood serum have been reported by Merkel[47] in the serum of young rabbits (blood taken shortly after birth) from mothers which were immunized during the period of gestation. He considers that it was a case of placental transmission. Bertarelli[48] immunized two dogs by subcutaneous injections of horse serum and of cow serum respectively; later he found a small amount of precipitins in the milk of each but found none in the blood serum of their young after four weeks nursing.

4. *Cytolysins.*—*a*) Bacteriolysins. Normal bacteriolytic bodies have been frequently demonstrated in different milks; likewise specific bacteriolytic substances have been demonstrated in the milk of immunized animals (Kraus[49]). No experimental work has been found demonstrating a transmission of specific bacteriolysins from the mother to the young. Clinically, several investigators have reported the relative bacteriolytic values of the mother's and infant's sera at the time of birth (Halban and Landsteiner[46]), the infant's serum showing a less amount of natural bacteriolysins than the mother's serum.

*b*) Hemolysins. Several investigators have reported upon the transmission of specific hemolysins from immunized mothers to offspring either through the placenta or the milk. Immunized rabbits appear to transmit the hemolysins to the fetus through the placenta (Bulloch,[15] Bertino[50]). Goats may or may not (Kraus,[44] Kreidl and Mandl[51]). In one case sheep failed to do so (Bertarelli[48]). Natural hemolysins have been reported as being present in some cases in the serum of infants at birth. The question of the transmission of hemolysins will be taken up later in this paper, and be fully considered.

5. *Opsonins.*—Turton and Appleton[52] compared the opsonic index of mother's and infant's sera in two cases (infants' ages, four and seven days) and found the infants' sera much lower than that of the mothers. Also they found, in comparative tests between the mother's serum and milk, that the latter was very much weaker in opsonins. It was not known whether the infants absorbed opsonin from the milk or not. This work has been in the main supported by other observers, both in human cases and by animal experimentation (v. Eisler and Sohma[53]).

6. *Allergin.*—It has been shown that female guinea-pigs which have been sensitized by injections of proteins, as horse serum, bear young which are also hypersensitive to the same serum during the early weeks of life (Rosenau and Anderson,[54] Anderson,[55] Gay and Southard[56]).

## B. ANTIBODIES OF POORLY DIFFERENTIATED TYPES.

1. *Bacterial, intracellular toxins.*—As is well known, a large group of pathogenic bacteria do not excrete specific, soluble toxins to any extent, so their poisonous prop-

erties are supposed to be mostly due to cellular proteins or so-called intracellular toxins. Active immunity may be developed by recovery from the disease, or by experimental vaccination with killed or attenuated cultures. Not much is known concerning this type of immunity, but it is probably quite complex in nature and dependent upon a number of factors. However, it has been experimentally proved that a mother immunized with certain members of this group may transmit a specific immunity to her young; for example (1) anthrax (Chauveau[3]); (2) swine erysipelas (Ehrlich[6]); or (3) tuberculosis. Behring[57] observed that a 14-day-old calf, born from a highly immunized cow (bovine tuberculosis), possessed considerable immunity against the organism. Behring thought that this immunity was acquired by the calf through the milk rather than by intrauterine transmission. He suggested the milk of specifically immunized cows as a means of combating tuberculosis in man, either as a preventive or a therapeutic measure. (4) Dysentery. De Blasi[58] actively immunized a rabbit against the dysentery bacillus by three inoculations of killed cultures during the first 18 days after birth of young. The young (two) were given on the 19th day surely fatal doses of the dysentery culture. Both remained alive, whereas controls died.

2. *Spirilla.—a) Sp. obermeieri.* Animals may be actively immunized against this organism by further injections of spirillar blood following a recovery from the infection; others may be passively immunized by the injections of serum from such animals. Novy and Knapp[59] found young rats, born from infected mothers, immune when tested after a few weeks. They looked upon this as an active immunity, due to intrauterine infection, since the spirilla may penetrate the placenta and reach the fetus. Therefore it remains an open question if an active immunity (intrauterine infection), or passive immunity (placenta, milk) from mothers' antibodies occurs in the young.

*b) Spirochaeta pallida* (syphilis). It is questionable whether a complete immunity ever follows in syphilitic patients after a clinical recovery from the disease. With the introduction of the complement fixation test in the diagnosis of syphilis, it has been found that many apparently healthy children born from luetic mothers give a positive reaction, while others, nonsyphilitic, may contract the disease by suckling a diseased mother (Ricketts and Dick[60]). This would prove that Profeta's Law* can no longer hold. It would appear that whatever immunity exists is slight. According to Thomsen,[61] a number of observers besides himself have found complement fixing substances in the milk from luetic women. It would be of interest to know if an infant taking such milk from a luetic mother would absorb these bodies in sufficient quantity as to give a positive reacting serum. It is still questionable whether these complement fixing bodies are true antibodies, or simply metabolic products brought about by pathological changes in the body of the patient during the course of the disease.

3. *Protozoa.—a)* Piroplasmosis (dog). Kleine and Möller[62] showed that immunized dogs transmit some immunity to their young against this disease.

*b)* Rabies. This disease is only tentatively placed under this division since the question of the protozoan nature of the so-called Negri bodies is still under discussion. Hoegyes[63] was one of the first workers to point out that actively immunized female dogs may transmit some immunity to their offspring. Konradi[64] carried out similar experiments upon dogs and in several cases with positive results. The work of Remlinger[65] with rabbits also supported those results.

4. *Ultramicroscopic organisms.*—Under this heading may be placed a group of

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* Profeta's Law assumed that a healthy child might be born from a luetic mother, and suckle the mother without contracting the disease.

contagious diseases which, on account of the constant failure to demonstrate definite specific microorganisms, has led many workers to consider the contagion in each case to be ultramicroscopic in size. (*a*) Sheep-pox (ovine). According to Burckhardt,[2] Richert immunized a large herd of sheep against ovine during the last six weeks of gestation. The lambs of these mothers were inoculated with ovine-lymph at the age of four to six weeks. None of them contracted the disease. A large number of controls showed pustules.

*b*) Foot and mouth disease. Loeffler[66] observed a calf born from a cow immunized against this disease, which proved to be immune against inoculations of the virus.

*c*) Small-pox and vaccinia. Since in the introductory portion of this paper small-pox has been briefly but sufficiently discussed, it will not be considered further. The work of Burckhardt[2] upon small-pox vaccine is of special interest since it was one of the first investigations upon the possible transfer of protective substances from mother to the child *in utero*. He vaccinated mothers during the last weeks of pregnancy, and then repeated the vaccinations upon the infants during the first days of their lives. He found in several that an apparent protection against the vaccinia had been acquired. Most of the control infants gave positive reactions. While these results were not conclusive, they offered suggestive data. In this connection it may prove of interest to note that in 1799, Hufeland (cited by Neumann[67]) was perhaps the first to suggest that the milk might exert a protective power for the child when secreted by a mother who had recently recovered from the disease. In this class of diseases, as has been previously pointed out, it is always a question whether the young have been passively immunized from the mother's antibodies, or if an active immunization has taken place from having had the disease before birth.

## III. DISCUSSION OF RESULTS.

In reviewing the literature on this subject, one is impressed by the broad scope of the studies, and the great amount of work which has been done. Even in the brief review just given, it is evident that the work on active immunity has been very productive of positive results. Also much work has been done by passively immunizing pregnant and nursing animals, but this has been purposely omitted in our résumé, since our studies cover only actively immunized mothers as influencing the offspring.

The possibility of an active intrauterine immunization on the part of the fetus must always be considered in connection with this type of experiment. Such may occur either through the passage of the living infectious agent or its products from the mother to the fetus. A distinction must be made between this condition and a passive immunization by means of the mother's antibodies through the placenta or through the milk to the young. In the diseases which are supposed to be due to ultramicroscopic organisms, the virus not infrequently seems to be able to pass the placenta and affect the fetus *in utero*. Where immune offspring are born from mothers which have passed through such a disease during pregnancy, caution must be exercised against accepting this as a true passive transmission of immunity (*in utero*) from the mother. To this we must add that animal experiments with certain living spirilla must be considered in the same light, since they may also pass the placenta and affect the fetus.

Most bacteria do not pass the intact placenta except under extraordinary conditions. Further, recent work (besides our own) would indicate that the fetus, or the very young animal, possesses only feeble reactive powers as to antibody production, when attempts have been made to actively immunize by means of prepared antigens. As shown by the foregoing review, fluids from fetuses aborted early in pregnancy by infected mothers usually gave negative results when tested for specific antibodies. Judging from these investigations we should expect the best results in the young from animals which have been actively immunized either against dead cells or toxins during the latter part of gestation. Even where such conditions were observed, positive results did not follow in every case. In part, the negative results may be accounted for by differences in experimental conditions, such as nature of antigen used, degree of active immunization attained, time of immunization relative to birth of young, character of resulting antibody, time when blood from offspring was first taken, etc. Further, many workers failed to make any distinction between the rôle played by placental or by milk transmission of antibodies; in many cases the blood of young was not drawn until after having the mother's colostrum and milk. In fact, blood samples in some instances were not taken until weeks after the birth of the young. Much of the literature cited in the above review covers very careful and painstaking investigations which have been carried out upon this subject. These studies prove that when the mother has been properly immunized during gestation and shows a high degree of immunity at the time of birth of young, some of this immunity (antibodies) may be passively transmitted to her young. This occurs either through the placenta before birth, or by means of the milk immediately after birth.

## IV. REPORT UPON ADDITIONAL WORK CONCERNING TRANSMISSION OF IMMUNITY FROM MOTHER TO OFFSPRING.

1. *Introductory remarks.*—As pointed out in the foregoing résumé, the passive transmission of antibodies to the offspring is practically established. This seems to hold for almost all classes of infectious organisms where a true active immunity (formation of antibodies) takes place in the mother's body. But the relative importance of the exact parts played by the two separate means of transmission—placenta or milk—has not been so fully decided. Some workers maintain that the placenta plays the greater rôle, while another but smaller group holds that antibodies do not pass the intact placenta. More work upon this subject, bearing in mind this phase of the question, seems desirable.

A large group of infectious diseases are induced by organisms which do not produce extracellular toxins (in culture) sufficient in amount or of such a nature that antitoxins can be produced by animal immunization. Recognizing the importance of this group of endotoxin-producing organisms, and their significance in disease, it was thought worth while to take up analogous studies which might throw more light upon the transmission of immunity to offspring. Since if by vaccinating the mother cautiously during pregnancy, the offspring also becomes immunized, a means is offered by which the young may be protected against those infections (pneumococcus, etc.) to which they are particularly susceptible during the first weeks of life. Up to the present time, we have no curative serum which offers much hope in these conditions. Active immunization (by vaccines) of the newly born against these organisms offers little encouragement and, in fact, may be followed by harmful sequelae. Not much is known concerning the nature of the immunity induced by actively immunizing animals against this type of organism. But perhaps in so far as the serum of such animals is concerned, the contained antibodies are quite complex in nature, and include antitoxins, bacteriolysins, opsonins, etc. Also there seems to be evidence that such a serum contains substances of which we know little, other than that they produce favorable results, exclusive of the bodies already mentioned. Since cytolysis must be one of the important factors in this type of immunity, the cytolysins may act as an index in carrying out experimental studies upon such immune sera.

As mentioned in the review, little has been done directly upon the bacteriolytic antibodies concerning their transmission from mother to offspring, but bacteriolysins have been found in milk from different animals. Most workers who have carried out studies along cytolytic lines have used lysins against red blood cells—the hemolysins.

In my own work, which is to follow, this means was adopted, as it appeared most advantageous. For instance, the test can be carried out completely in test tubes, and, under proper technic, with a high degree of accuracy. Also, test animals, as necessary in diphtheria or tetanus antitoxin work, can be entirely eliminated. As compared with bacteriolytic work the hemolytic tests are much more rapidly performed and probably more easily graded.

2. *Review of work reported upon transmission of hemolytic antibodies.*—After a careful search through the literature I find that several investigators have already reported work concerning the transmission of hemolysins from mother to offspring. However, some of this work escaped my attention until after my own work was well under way. Although briefly mentioned in the previous general review, these reports will now be considered more fully.

Kraus,[44] in 1901, reported his work upon the possible transmission of immune hemagglutinins and immune hemolysins from mother to offspring. He found that rabbits immunized by repeated subcutaneous injections of defibrinated dog's blood showed in their milk specific hemagglutinins, but these bodies were not carried over to the blood of the suckling. For the study of hemolysins, he immunized goats by injecting with sheep blood. No hemolysin could be found in the milk, so he concluded that these immune bodies were not excreted by the mammary glands, although the blood serum of the same animal showed quite definite amounts of the hemolysin. However, he found that the young born from one of the immunized goats showed a low degree of immunity (hemolysin) which was transient. Kraus thought that this was acquired before birth, and considered it a passive immunization from an actively immunized mother. He gives no definite data concerning the hemolytic tests of the serum but apparently he added no normal complementing serum, but depended upon the small amount of complement which was present in the immune serum used in the test. As is well known, goat serum is weak in hemolytic complement, and therefore will not bring out the highest efficiency of the contained immune hemolysin, even when present in large amounts. As a result, his data show the hemolytic sera to be of very low value. But in the milk tests he added normal goat serum, as complement, since he thought it possible that the milk glands might not let the complement through, consequently no activation of the immune body could take place in case it were present. In view of negative results, he concluded that immune body was not present in the milk. He did other experiments with goats' milk which indicated that it was deficient in complement.

Following this, Bulloch,[15] in 1902, took up the study of transmission of hemolysin from parent to offspring, using rabbits immunized against blood cells of the ox. He considered not only the rôle of the immunized mother, but also that of the father from the standpoint of heredity, and obtained results which, on the whole, supported the early work of Ehrlich. But of special interest to us are his findings concerning the transmission of specific hemolysin from mother to the young. When the blood injections were given to the gravid animals, pregnancy was interrupted, but the fetal fluids were found strongly hemolytic. In those cases in which the injections were made prior to conception, the young were reared, and in one case the inactivated serum of the young was equal to that of the mother in hemolytic power, while the milk was only one-third as active as the mother's serum. In another case the serum of the young, which was found dead on the day of birth, was one-half the value of that of the mother, while the milk was about one-fourth of that value. Bulloch agreed with Kraus that no complement passed out with the milk,* but showed in opposition to Kraus that hemolytic immune body is excreted by the milk glands. He used fresh guinea-pig serum as complement in all of his tests.

Kreidl and Mandl[51] (1904), in a preliminary report, stated that goats injected with beef blood might lead to a passive immunization of the fetus, but from their tests concluded that in the majority of cases, the specific hemolysins do not pass from mother to fetus through the placenta. They considered individual variations to be responsible for the lack of uniformity in their results. No definite experimental data are given in this report. They also consider the transmission of immune hemolysins from the fetus over to the mother, but as their results are not fully conclusive, they will not be discussed in this connection.

Bertino[50] (1905) states that in rabbits, when the immunization of mother has taken place before conception, the lysins do not pass over to the fetus, nor to young during the suckling period, but when the immunization is carried out during pregnancy, there is a transmission of the specific antibodies to the fetus. Since the original article was not available these results are quoted from an abstract (*Folia Haematologica*, 1905, 2, p. 624), and I am unable to give further details.

Bertarelli[48] (1906) reports that he found a small amount of specific hemolysins in the milk of a sheep immunized against hen's blood, but found none in the blood of the young. This immunization was carried out by repeated injections of the blood during the last weeks of pregnancy and the first weeks after birth of lambs.

Considering the report just presented, it would appear that hemolysins may be transmitted through the placenta to the fetus *in utero*, especially in the examples cited by Bulloch. But objections may be raised as to the validity of the findings from aborted fetuses, since in those cases a placental lesion could exist which would permit a direct connection between the maternal and fetal circulation. Apparently the ox blood injected seemed to exert a toxic action upon rabbits. It would be difficult to exclude the influence of absorption of hemolysins from milk previous to time blood samples were taken from young, in some of the cases. The question of animal species may enter largely in determining the results. The meager data available in Bertino's work upon rabbits would support the statement of Bulloch. Perhaps larger animals, as goats or sheep, do not permit this class of antibodies to pass the placenta so readily as some of the smaller animals.

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* Lane-Claypon (*Jour. Path. and Bact.*, 1908–9, 13, p. 34) claims to have demonstrated complement in fresh milk (in about 1/10 value of the individual's serum) as well as natural immune bodies, by the addition of the "ox colloid" of Bordet. Others have also shown the presence of the hemolytic complement in milk (Pfaundler and Moro; *Ztschr. f. exper. Path. u. Therap.*, 1907, 4, p. 451).

Another important point, which must be considered in such experiments involving specific hemolysins, is the presence of natural hemolysins (for the blood used) in the blood serum of the mother, and their development in the body of the fetus, or possible passage over from the mother's circulation. Much variation in this respect may exist between different individuals of the same species. It is necessary carefully to control adult serum as to content of natural immune bodies against the particular blood to be used for immunizing purposes, before any injections are made.

3. *Choice of experimental animals and outline of technic.*—In the present work goats were selected for experimental animals since they are more highly developed, rugged, and withstand experimental handling (injections, bleedings, etc.) very readily, and usually give a bountiful supply of milk. Sheep corpuscles were used for immunizing purposes; their advantage being that goat serum seldom shows the presence of more than a small content of natural hemolysins, if any, against these corpuscles. Goats readily respond to the blood injections by specific hemolysin production. This immunity may be raised to a high degree by repeated injections of the blood, while practically no toxic action is exerted upon the animal. Moreover, only a small amount of hemagglutinins are formed by goats against sheep corpuscles; hemagglutinins when present in large amounts, in a hemolytic serum, cause a marked disturbance in the hemolytic reaction.

In my experiments, the technic followed was similar in all cases, and may be briefly outlined as follows:

1. Preparation of sheep's blood for injection, or for hemolytic test: Blood was drawn from the jugular vein of normal sheep by puncturing the skin and vessel wall

with a large hollow needle and collecting the flow in a sterile bottle containing a coil of wire. It was defibrinated by shaking, then placed in tubes, centrifuged, the serum removed, and then the corpuscles were washed three to four times in 0.9 per cent NaCl solution until they were practically free from all serum.

2. The injections of blood in these experiments were all made subcutaneously. If the volume was very large, it was divided and injected into the animal in several places.

3. Collections of blood samples. Bleedings were made from the jugular vein. In case of adult, a hollow needle was used, but from kids, a few c.c. were drawn by means of a hypodermic syringe. In each case blood was placed in a test tube and allowed to clot out. When the serum had separated, it was removed and placed in small sterile bottles.

4. Milk samples had 10 per cent by volume of 1:1,000 formaldehyde in 0.9 per cent NaCl solution added. Preliminary tests showed that this strength of formaldehyde solution exerted an antiseptic action, while it had little if any influence upon hemolytic antibodies in milk. The milk samples were then placed in bottles for storage. Both serum and milk samples were stored in ice-box where they were kept until the entire series covering the experiment were collected. Tests were done with the fluid portion which was removed from under the cream layer, after this had separated. The first colostrum, which was heavy and tenacious, failed to separate a cream layer, so was used as a whole.

5. When a full series of samples of sera and milks for a given experiment were collected, all were tested at the same time, thereby using the same blood suspension, complement, technic, etc., which made the results much more comparable. Preliminary tests were first made to get relative values of the samples. The serum became "inactivated" by aging; no heat was applied in any case. The ordinary test tube method for the determination of specific hemolytic immune body* was employed.

For complement, in the earlier experiments, fresh rabbit serum (0.20 c.c. per tube) was used; later, fresh serum from guinea-pigs (0.10 c.c. per tube) was used. This proved to be more efficient, and was used in all tests which are cited except when otherwise noted.

The scheme followed for the tests was to arrange a series of test tubes for each sample; the serum or milk which was to be tested was first accurately measured in decreasing amounts directly from sample or from the proper dilution in physiological (0.9 per cent) salt solution dilution, into a series of test tubes. Then physiological salt solution was added to bring the volume of each tube up to 1.0 c.c. To this was added the given volume of complementing serum, then 2 c.c. of 7.5 per cent suspension of sheep corpuscles. Immediately the tubes with the mixtures were thoroughly shaken, then placed in the incubator (36°–37° C.). After one hour they were removed, again thoroughly shaken and placed in the ice-box until any remaining, intact corpuscles settled to the bottom of the test tube. After the corpuscles had sedimented, a transparent fluid remained above, varying from a deep red color to a water white, depending upon the presence or absence of hemolysis. The results of the tests were read by making equi-colorimetric readings throughout the various series of samples by comparing with a "standard color" tube which was made by "laking" a given volume of the blood corpuscles in distilled water.

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*The term "hemolytic immune body" is used in this report as a more suitable equivalent than "substance sensibilisatrice" (Bordet), or "intermediary body" "amboceptor" (Ehrlich) for the biological reaction product of the organism against injected alien blood cells.

4. *Groups of experiments.*—For convenience the experiments will be considered under four groups; the first two cover the main tests upon the transmission of hemolysins from the mother to the young, while the remaining groups cover certain tests which have a general bearing upon the subject.

In the first group the female goats were actively immunized against sheep corpuscles by the method outlined. Each experiment was a comparison of the relative content of specific hemolysins in the mother's serum, the serum of her own young, and of her colostrum or milk. No "cross nursing or feeding" of young was undertaken. The tests are not given in perfect chronological order, but each experiment will be given a number for reference purposes, and these will run consecutively throughout the report. In order to avoid repetition, typical experiments will be reported in full; others falling under the same head will be briefly described.

*a*) Active immunization of the adult before birth of the young, either during the period of gestation, or before conception had taken place.

### SERIES 1. PRELIMINARY EXPERIMENTS.

To obtain data concerning the possible transmission of hemolysins from mother goats to their young, preliminary experiments were undertaken. Since the results proved instructive from several standpoints, a part of the data will be submitted. In this series, pregnant animals were immunized during the last weeks of gestation or immediately before the birth of the young. Blood samples were taken from the adults before the injections were started, and then at short intervals during the course of immunization. On day of birth of the young, blood samples from the mother and young, and a sample of the mother's colostrum were taken. Following this, samples were taken at intervals for two and a half to five weeks or even longer.

*Experiment 1. Goat 4 (normal; pregnant).*—During the course of nine days this animal received six subcutaneous injections (increasing doses) of washed sheep corpuscles—a total of about 400 c.c. On the morning of the 12th day after the last injection it was found that she had given birth to two normal kids; one was removed from the mother about noon, and placed on cow's milk although it had undoubtedly freely suckled its mother. Late in the afternoon blood samples were taken from each kid. During the same forenoon, both blood and colostrum-milk samples had been taken from the mother. Test samples from each were taken at intervals for over two weeks following the birth of the young. Comparative tests (regular technic, 0.20 c.c. rabbit serum for complement) were made upon all of the samples at the same time and the following results were given:

| Time | Mother's Serum | Colostrum-Milk | Kid "A" Serum (Mother's Milk) | Kid "B" Serum (Cow's Milk) |
|---|---|---|---|---|
| Day of birth of young........ | 0.06 c.c.* | 0.02–0.03 c.c. | 0.035–0.04 c.c. | 0.045 c.c. |
| 1st day after birth........ | ...... | 0.20–0.30 " | 0.04 " | 0.05 " |
| 2d " " " ........ | 0.055 " | Traces | 0.045 " | 0.08 " |
| 4th " " " ........ | 0.06 " | 0 | 0.06 " | 0.08 " |
| 6th " " " ........ | 0.035 " | 0 | 0.08 " | 0.09 " |
| 10th " " " ........ | 0.047 " | 0.20 c.c. | 0.10 " | 0.175 " |
| 14th " " " ........ | 0.075 " | 0 | 0.15 " | 0.20 " |

*When comparisons are made between the samples it must be understood that the potency value is inverse to the quantity value. The numbers indicate the volume of each sample which was required to produce a given degree (constant) hemolysis in that test.

Further tests showed that the antibody content of the blood in both kids continued to fall, more or less in parallel. The adult's serum showed its highest value on the sixth day after the birth of the young (18th day after the last injection of blood), then gradually declined. The milk showed transiently (eighth to 10th day) demonstrable amounts of antibodies, following shortly after the adult serum showed its greatest concentration in antibodies.

*Experiment 2. Goat 14 (normal; pregnant).*—Two subcutaneous injections of washed sheep corpuscles were given on consecutive days: first day, 125 c.c.; second day, about 140 c.c. Twenty-one days after the last injection, the mother was found in the morning with one normal kid which was allowed to remain with her. At noon blood samples were taken from both and also a sample of the mother's colostrum-milk. The kid had suckled and stomach seemed full at time the bleeding was made. During the course of immunization, blood samples were collected from the mother, and this was continued with the addition of milk samples at intervals for some time after parturition. Likewise, blood samples were taken from the kid, and finally all samples were tested at the same time to get the relative hemolytic values. The adult's serum showed its maximum hemolytic value (0.0075 c.c.) 13 days before birth of young. From this high value it rapidly receded until on the day of birth of young it gave a comparative value of only 0.035 c.c. It remained fairly constant at the same level for about one week, then gradually fell. The colostrum-milk showed a value of 0.05–0.06 c.c. on day of birth of young; the antibodies rapidly disappeared from the milk so that by the second day after the birth the test failed to show their presence. The kid's serum from blood samples taken on day of birth showed a value of 0.017 c.c., while a sample taken a few days later showed about twice this value, then gradually receded. This would lead us to believe that gastro-intestinal absorption of hemolysins from the milk is an important factor in the very young animal. The first blood sample was taken from the kid a few hours after birth and although it had freely fed, the absorption of antibodies was still incomplete but the assimilation from the digestive tract continued after this time, as was indicated by higher value of its blood serum taken later. This question will be taken up again and more fully discussed.

*Experiment 3. Goat 11 (normal; pregnant).*—This animal was given subcutaneous injections of washed sheep corpuscles spaced three days apart—three in all—100 c.c., 75 c.c., and 75 c.c. A kid was born during the evening or night following the last injection; it was allowed to remain with the mother. The mother's serum showed no definite immune bodies on day of birth of the kid. Two days later blood samples from both were drawn and a milk sample was taken from the mother; this was repeated on alternate days for several weeks. All were tested at the same time, using 0.20 c.c. of rabbit's serum for complement. The mother's serum showed its highest value in specific hemolysins on the 10th day after last injection of blood cells, then gradually receded from this point. The milk showed a low antibody content; the mother's serum and milk reached their highest concentration simultaneously (serum 0.0035 c.c., milk 0.20–0.25 c.c.). The kid's serum showed questionable traces of specific hemolysin on the fourth and sixth days after birth but none later, although the blood serum was watched for several weeks. This test shows that an immunity to erythrocytes arising during the period of lactation has an influence upon the milk, but that the antibody content is less than 1 per cent of that of blood. Further, a kid at this age must receive much higher quantities of antibodies through the milk, in order to absorb a sufficient quantity to be easily demonstrable in its blood serum. This will be more fully supported by results of other tests which are to follow.

These preliminary tests indicate that mothers actively immunized against the erythrocytes during the latter part of pregnancy can transmit these antibodies to the offspring. But when the immunization has progressed only slightly, that is, no antibodies are demonstrable in the blood until after the birth of the young, the young fail to show more than a trace of antibodies in their blood. Presumably these are acquired through the milk. Probably in all cases this transmission was passive, and took place either through the placenta or the colostrum-milk, or both. I was led to think from these early tests that of the two factors each was about equal in importance. It was found that the colostrum-milk was rich in specific antibodies when the females were immunized before parturition. Also, the kids showed a rise of antibody content in their blood, as they took and absorbed this colostrum, which rapidly reached a maximum and then decreased slowly, as in passive immunization. Further, it was observed that an animal which received first blood injection only a few days before and the last on day of birth of young, did not influence the offspring to any appreciable extent; the milk showed a very low antibody content.

### SERIES 2. SPECIAL EXPERIMENTS.

A series of experiments were undertaken by which the relative importance of the two factors—placenta and colostrum-milk—in the transmission of hemolysins to the young could be determined. For this purpose goats were actively immunized against sheep erythrocytes in the regular manner either during the course of gestation, or before conception had taken place. Blood samples were taken from each goat before the injections were started, and at short intervals during the course of immunization. To exclude the possibility of the young getting any milk immediately following birth, the ends of the mother's nipples were previously sealed with collodion. After birth of the young, blood samples were taken; the collodion seals were removed from the mother's nipples, a colostrum sample taken, then the newly born animal, which was assigned to the mother, was allowed to suckle. In case twins came, one was never allowed to suckle the mother but was put directly upon cow's milk as a control. Test samples were taken from mother and young at frequent intervals for some time following the birth. In all tests in this series 0.10 c.c. fresh guinea-pig serum (per tube) was used as complement.

*Experiment 4. Goat 29 (normal; pregnant).*—Six subcutaneous injections of sheep erythrocytes were spaced over 16 days in gradually increased doses, a total of 450 c.c. blood cells. On the 34th day after the last injection, the animal gave birth to two kids. Blood samples were taken from both before they were allowed any milk. One was removed and placed upon cow's milk, while the other was allowed to take mother's colostrum after the collodion seals were removed and a sample drawn. The serum from a blood sample taken from the mother before first injection of sheep blood, showed a definite amount of normal hemolysin (0.10 c.c.) against the sheep corpuscles used in the test. During the course of the immunization the mother's serum showed the highest antibody concentration (0.008 c.c.) nine days after the last injection; the content rapidly fell so that on day of birth of young (34th day after last injection) it was quite low. Since 0.10 c.c. of the mother's serum showed a definite amount of natural hemolysins against the sheep corpuscles, no greater volume of the test samples were used. The results of the comparative tests are as follows:

| Time | Adult's Serum | Colostrum-Milk | Kid "A" Serum (Mother's Milk) | Kid "B" Serum (Cow's Milk) |
|---|---|---|---|---|
| Day of birth of young........ | 0.06 c.c. | 0.018 c.c. | 0 | 0 |
| 1st day after birth of young.. | ..... | 0.08 " | 0.048 c.c. | 0 |
| 2d " " " " " .. | 0.070 " | ? | 0.045 " | 0 |
| 4th " " " " " .. | ..... | 0 | 0.050 " | 0 |
| 7th " " " " " .. | ..... | 0 | 0.058 " | 0 |
| 10th " " " " " .. | 0.10 " | 0 | 0.060 " | 0 |
| 13th " " " " " .. | Trace | 0 | 0.060 " | 0 |
| 17th " " " " " .. | Trace | 0 | 0.075 " | 0 |

It is shown by the above data that the adult rapidly lost its specific hemolysins—even the natural hemolysin seemed to have diminished. According to the test, neither of the kids showed any trace of hemolysin in 0.10 c.c. of their sera taken on the day of birth; the one on cow's milk continued negative, while the one with the mother acquired, by absorption from the colostrum-milk, a definite hemolytic content in its blood. The colostrum showed a relatively high amount of hemolysin at time of birth of young, but this rapidly diminished until the test failed to show its presence.

*Experiment 5. Goat 31 (normal; pregnant).*—Received every third day an injection of sheep corpuscles until six were given—total volume injected was 390 c.c. Samples were taken both before blood injections were made and at intervals during the immunization. Six days after the last injection, the animal was found with two normal young. Since the mother's nipples were sealed, neither kid had any milk; blood samples were taken from each, then one was removed and placed on cow's milk; the other was allowed to suckle mother after the collodion seal was removed and a sample of colostrum was taken. Unfortunately this experiment was of short duration, since the kid with mother became ill (probably from cold) the second day after birth, and experiment had to be discontinued. However, the results are of sufficient interest to be briefly tabulated here.

| Time | Mother's Serum | Colostrum-Milk | Kid "A" Serum (Mother's Colostrum) | Kid "B" Serum (Cow's Milk) |
|---|---|---|---|---|
| Day of birth of young........ | 0.0075 c.c. | 0.015–0.020 c.c. | 0 | 0 |
| 1st day after birth........... | ...... | 0.035–0.040 " | 0.05 c.c. | 0 |
| 2d " " " ........... | 0.0097 " | Trace | ...... | 0 |

This test shows that while both blood serum and colostrum from mother contains a high antibody content, neither kid had sufficient antibody in blood at birth to give even a trace of hemolysin when 0.10 c.c. serum was used, but the one which had taken mother's colostrum on day of birth, showed a fairly high amount of the specific antibodies in its serum on the following day.

*Experiment 6. Goat 15 (pregnant).*—This animal had been immunized (sheep erythrocytes) five months previously, but at the time this experiment was undertaken no specific hemolysins were demonstrable in the blood serum. Again subcutaneous injections of sheep blood cells in gradually increasing doses (six) were given, three to four days apart, making a total of 250 c.c. in all. A test showed that the serum reached its highest value (0.001 c.c.) on the seventh day after the last injection, but at the time of birth of kid it had greatly decreased (0.020–0.025 c.c.). One normal kid was born 115 days after the last injection. Shortly after birth a blood sample was taken from the kid; the seals were removed from the mother's nipples and a sample of colostrum was drawn. Then the kid was allowed to remain with the mother and suckle. On testing the samples the mother's serum showed a relatively low content (0.020–0.025 c.c.) of hemolysin, which remained quite constant for some days after the birth of young. The colostrum while scant was especially high in its hemolytic content (0.009 c.c.) on day of birth. On the following day the milk showed a very low test (0.15 c.c.) and was entirely negative by the end of the week. A sample of 0.30 c.c. of the kid's serum at birth did not show a trace of hemolysin by test, so probably little if any passed the placenta from the mother. On the second day after having suckled, only a trace of hemolysins could be demonstrated in the kid's serum; this can be explained in part by the known fact that the mother had only a small amount of colostrum, the volume of milk secreted was scant for several days, and the kid showed signs of hunger and under-nourishment.

*Experiment 7. Goat 6 (pregnant).*—It may be of interest to record in this connection an observation made on another goat which might be classed with this series. The mother showed signs of oncoming labor during the late afternoon, so both blood and colostrum samples were taken. Next morning it was found that the animal had given birth to two large (weight four lbs. each), apparently fully developed young which were dead. Nothing in particular was shown on autopsy excepting some subcutaneous edema in each; some bloody fluid was found, in the thoracic cavity, which was removed and saved for test; the heart and large vessels were fairly well filled with partially clotted blood which was removed and the serum collected; lungs, nondilated, on section showed nothing unusual; the other organs appeared normal. Possibly a large bleeding (between 300–400 c.c.) which was taken from the mother one week before, might have had some influence upon the welfare of the young. The weight of the adult, on day following the birth of young, was 83 lbs. Upon centrifuging the fluid from the thorax, and the blood from heart and great vessels, it was found that in one kid the fluids came out quite clear, while those from the other were rather deeply colored by the presence of hemoglobin. We shall therefore consider the tests made upon the clearer fluids from the one kid. The following comparative values were shown: Mother's serum, 0.0025 c.c.; Colostrum, 0.0015–0.0020 c.c.; Kid—the fluids from the blood and from the thorax failed to show a definite hemolysis in amounts up to 0.20 c.c.; a very slight tint was observed but it is questionable if this were due to specific antibodies or traces of hemoglobin in the sample.

This adult was unique in the sense that she seemed to retain a very high antibody content in her blood long after the last blood cell injection. The last blood injection was given about six months (170 days) before birth of the young. Most animals lose their antibodies much more quickly; few show them in appreciable quantities after six months. This animal was immunized before conception had taken place, since the period of gestation in goats is stated to be five months. If the hemolytic immune bodies passed through the placenta to the fetus in this case, the amount was very small.

From the experimental evidence given in this last series of experiments it would appear that the colostrum plays a greater rôle in the transmission of hemolytic immunity to the offspring than the placenta—the latter probably transmits little if any appreciable amount of hemolytic immune body, to the fetus *in utero*.

*b*) Active immunization of the adult following immediately or shortly after birth of young.—A number of workers have succeeded in highly immunizing female animals, during the period of lactation, against toxins and getting the antibody in the milk in a relatively high concentration. Since it has been proved that the milk is an important factor in transmitting antibodies to the suckling during the first days of life through gastro-intestinal absorption, it was thought worth while to undertake similar experiments at later periods in connection with our studies.

### SERIES 3. ANIMALS NORMAL AT TIME OF PARTURITION.

*Experiment 8. Goat 8 (normal). Preliminary test.*—This animal gave birth to one normal kid which was allowed to remain with and suckle mother. Blood samples were taken from the mother and kid, then the mother received a subcutaneous injection of 75 c.c. of sheep corpuscles. Following this, 100 c.c. corpuscles were given daily for three consecutive days—a total of 375 c.c. Blood samples were drawn from the mother and kid on alternate days for two weeks, after which samples were taken every third day for some time longer. In this experiment no milk samples were taken during the first weeks as the supply was scant; the serum samples were tested at the same time using 0.20 c.c. rabbit serum per tube for complement. It was found that antibodies did not appear in definite amounts in the mother's serum until five days after the last blood injection, then they rapidly increased until the 11th day, when the highest concentration (0.0035 c.c.) was found; the antibodies slowly declined until on the 46th day after last injection the value had fallen to 0.037 c.c. Serum samples from the kid when tested in amounts as high as 0.5 c.c. failed to show positive results. It is questionable if any appreciable amount of hemolysin was excreted in the milk when the mother's serum was at its greatest concentration. If so, the kid at that age failed to absorb (unchanged) a sufficient quantity to give a definite hemolytic reaction in the test.

*Experiments 9 and 10.*—Similar experiments were performed with goats 4 and 5, with the exception that milk samples were taken throughout and tested at the same time as the blood serum samples of the respective mother and kid. In neither case did we succeed in getting very high immunity in the mother animals, and it was found that an appreciable amount of antibody failed to pass over in the milk. Naturally the serum of the kids did not show antibodies.

These experiments proved quite unsatisfactory. It appears that an exceedingly high immunization of the female is necessary before a fairly high antibody content occurs in the milk. In neither of the two goats last mentioned was the degree of immunization sufficient to cause a demonstrable overflow of antibodies in their milk.

An obvious objection to the active immunization of normal females immediately after birth of young is the necessary delay before the production of antibodies in high concentration in the mother's body, and their appearance in the milk. In the meanwhile the young animal becomes more mature; its digestive powers are more pronounced, and probably anatomical changes occur in the gastro-intestinal mucosa which retard or prevent the absorption of the unchanged antibodies.

### SERIES 4. ANIMALS PARTLY IMMUNIZED AT TIME OF PARTURITION.

In order partly to overcome certain objections to which attention has just been directed, animals which had been previously immunized and still showed a low antibody content in their blood at the time of birth of the young, were reimmunized by more blood injections following parturition. In this way it was hoped that a high immunity could be induced in the mother, and more quickly than was possible in the strictly normal animals, and that the milk would either remain high or increase in immune body value. Possibly kids receiving such milk in quantity during the first weeks of life would be able to absorb the immune body unchanged, and as a result their sera would remain about constant or even show some decided rise in the hemolysins. In opposition, other factors enter, such as (*a*) animal growth (increase in body fluid), (*b*) loss through blood samples withdrawn, and the (*c*) metabolic destruction of antibodies. Therefore, such experiments should be well controlled.

*Experiment 11. Goat 6 (previously immunized).*—Found in the forenoon with normal twins, and as they had suckled, only a small sample of milk could be obtained from the mother. Both remained with mother until late in the afternoon. The mother was given a large injection of washed sheep corpuscles the same evening.* The blood injections were repeated every second day until four in all were given—a total of 425 c.c. Blood samples (adult and kid) and milk were taken for several weeks. All were tested simultaneously. The results of the test may be tabulated as follows:

| Time | Mother's Serum | Milk | Kid "A" Serum (Mother's Milk) | Kid "B" Serum (Cow's Milk) |
|---|---|---|---|---|
| Day of birth of young........ | 0.0080 c.c. | 0.05–0.06 c.c. | 0.002 c.c. | 0.0035 c.c. |
| 2d day after birth of young.. | 0.0077 " | 0.27 c.c. | 0.002 " | 0.0030 " |
| 4th " " " " " .. | 0.0040 " | 0.16 " | 0.003 " | 0.0035 " |
| 6th " " " " " .. | 0.0020 " | 0.08 " | 0.003 " | 0.0040 " |
| 10th " " " " " .. | **0.0009** " | 0.05 " | 0.004 " | 0.005 " |
| 12th " " " " " .. | 0.00098 " | **0.045** " | 0.0042 " | 0.005 " |
| 16th " " " " " .. | 0.0012 " | 0.05 " | 0.006 " | 0.007 " |
| 22d " " " " " .. | 0.0017 " | 0.15+ " | 0.0078 " | ...... " |
| 28th " " " " " .. | 0.0022 " | 0.15+ " | 0.009 " | 0.019 " |
| 34th " " " " " .. | 0.0027 " | 0.18 " | 0.015 " | 0.03 " |
| 40th " " " " " .. | 0.003 " | 0.27 " | 0.022 " | 0.045 " |

(When 45 days old, Kid "A" weighed 14½ lbs., and Kid "B," 17 lbs.)

As evident from the above data, the kid which was with the mother and getting a bountiful supply of milk containing large amounts of immune body, showed in its blood serum an antibody content practically running parallel with that of a control (brother) which was fed upon cow's milk after the first day following birth.

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* At the same time this animal received injections of killed cultures of the colon bacillus in small but increasing doses on days alternating with the blood injections. These injections were continued over two weeks without any harmful effects in so far as noted.

The ratio of weight between the two kids probably ran roughly parallel throughout the test. So it would appear, at least in this instance, that not many of the antibodies pass over unchanged to the circulation from the gastro-intestinal tract of a suckling animal after the first days following birth.

This experiment would suggest that even when we can get an animal highly immunized and the milk shows the presence of considerable antibodies, hardly an appreciable amount is absorbed unchanged from the digestive tract by the suckling after the first days of life. These results were supported by other observations, but in experiments where a control twin was not available.

*c*) Active immunization of newly born or older kids against foreign blood cells.

### SERIES 5. BLOOD INJECTIONS INTO KIDS.

In connection with our studies where mothers received very large injections of blood during gestation, especially in the very last weeks, we must consider the possibility of soluble antigens passing the placenta to the fetus. Bang and Forssman[68] state that ox corpuscles gave an ether soluble fraction which possessed antigenic properties when injected into suitable animals. It is possible, therefore, that in the body also similar soluble products are liberated in quantity and eventually reach the blood stream from the large subcutaneous blood injections. Hektoen and Carlson[69] have shown by transfusion experiments with dogs, which had been previously injected (intravenous) with small amounts of goat's blood cells, that after several hours the antigen is fixed in the body of the injected animal, and not transmitted in sufficient quantity to the normal recipient to cause specific antibody production in the latter. But when large blood injections were given to a donor, then later a direct transfusion was made to a normal animal, the recipient as well as donor produced antibodies in due course of time. This showed that the body's ability to fix the formed antigen was limited. Therefore the possibility exists that, from large subcutaneous blood injections into gravid goats, soluble antigen might eventually reach the fetus. The question arises, If the fetus receives antigen shortly before birth, has it the power to react and form antibodies, or may it retain the antigen and form antibodies shortly after birth? Any attempt directly to inject the fetus* *in utero* is necessarily surrounded by numerous technical difficulties. Moreover, the results thus derived would be justly subject to criticism. A few experiments were carried out on kids at different periods after their birth to test their reactive ability against sheep erythrocytes. The results may throw some light upon this side of the immunity problem.

*Experiment 12. Kid 96 B (newly born)*.—This kid was a twin born from a mother which had been immunized against sheep corpuscles, over five months before parturition. The mother's blood taken six days before birth of the young showed a trace of hemolytic immune body in 0.10 c.c. serum, while a sample taken on the day of birth failed to show any hemolysin in like amount of serum. The colostrum on day of birth of young showed a small amount of immune body in 0.10 c.c., but the milk on the following day was negative. A blood sample was taken from the kid before it was allowed to suckle, then it was placed with the mother. The same evening 12.5 c.c. of sheep corpuscles were injected into the kid subcutaneously and the next afternoon another injection of the same amount of corpuscles was given. Blood samples (eight) were taken at intervals during the 15 days following the last injection. On testing it was found that 0.10 c.c. serum from blood samples taken the second and third days after birth showed a small amount of hemolysin, but this practically disappeared and soon showed only as a trace. Since the kid's blood sample taken before suckling showed no hemolytic action in the same amount, one must look to the colostrum as a source of its antibody on second and third days. The tests of samples, covering two weeks following last blood injection, failed to show more than a trace of hemolysis at any time, but perhaps this trace was slightly greater at the end of the second week. Adults usually show specific hemolysin production by the end of the first week after injection, and often this is at a maximum on the ninth to 12th day. We must conclude that the young in this case produced hemolysins only to a very slight extent (minute quantity) in response to the injection of sheep blood corpuscles in relatively large amounts.

*Experiment 13. Kid 15 B (10 days old)*.—This kid was also born from an immunized mother, but failed to show any appreciable amount of specific hemolysins in its blood serum at the end of the first week after birth. On the 10th day after its birth the kid was given a subcutaneous injection of 10 c.c. of sheep blood corpuscles and an equal dose was given in the same way on the 12th day. Samples were collected at intervals for three weeks after the last injection. By the test,† all were negative (0.10 c.c.) until the ninth day, when a slight trace of hemolysin was shown; on the 12th day this was somewhat increased, a definite but slight hemolysis; 16th day, questionable trace; 20th day, 0.10 c.c. gave negative results. This would indicate that a slight but transient immunity reaction took place against the sheep blood cells.

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* Kreidl and Mandl (*loc. cit.*) attempted injection of the fetus (goats) *in utero*, but were unsuccessful in the outcome in practically every case.

† Used the regular technic excepting 2.0 c.c. of a 5 per cent instead of a 7.5 per cent suspension of sheep erythrocytes were added per tube.

*Experiment 14. 68 B (18 days old).*—The mother of this kid had been immunized against sheep blood cells, some time previously. It was removed from the mother on day of birth and fed during the first days upon goat's milk and then later upon cow's milk. On the 18th day after its birth, 20 c.c. sheep blood cells were injected into it subcutaneously and two days later an equal volume was given in the same way. Blood samples were taken during three weeks following the last injection. Testing by the regular technic, it was found that 0.10 c.c. of serum showed a trace of antibody, on the 18th day after birth (first injection of blood). Probably it got colostrum, which contained immune body, on day of birth. These antibodies quickly disappeared and further samples were negative in amount tested, until six days after last blood injection, when a slight trace again appeared and gradually increased so that on the 12th day after the last injection of blood, a slight but definite hemolysis was produced by 0.10 c.c. of serum; this had again practically disappeared within a week. This experiment indicates that only a very slight immunity reaction occurs in a kid after it has reached the age of 18 to 20 days.

If we are permitted to draw any conclusions from these few experiments we should say that the newly born animal has only slight ability to form hemolysins during the first days following birth. Consequently we cannot assume that the fetus *in utero* has greater power, even if antigen should reach it through the placenta. But, on the other hand, it is more probable that the fetus has even lesser power to form immunity reaction product than the newly born. This seems to be a property which is only gradually acquired by the growing young, as they adjust themselves to their environment in the external world. The work of several investigators (Schkarin,[70] Moll[71]) would seem to substantiate the view that the organism of very young infants or animals have only a slight ability for antibody production.

*d*) Gastro-intestinal absorption of antibodies.

### SERIES 6. FEEDING OF HEMOLYTIC SERUM TO NEWLY BORN KIDS.

As apparent from the data submitted, the newly born kid readily absorbs in an unchanged condition the specific hemolytic immune body from colostrum through the gastro-intestinal tract. These immune bodies do not appear in the kid's blood serum unless present in considerable amounts in the ingested colostrum. However, the amount in colostrum-milk may be comparatively low and still cause a decided increase in hemolytic value of the suckling's blood, if the total volume taken is large. The question suggested itself, Was this free absorption of immune body taken through the milk-colostrum peculiar to the combination? Or, are the immune bodies readily absorbed by the newly born kid, when fed hemolytic serum, irrespective of the nature of the medium in which they are present? As we had an opportunity, this question was put to test in two cases by feeding newly born kids with homologous serum (goat) which contained specific hemolysins. The kids were taken away from the mothers immediately after birth and were never allowed to suckle. Owing to the lack of animals at the time this investigation was in progress, exact quantitative experiments upon this question, unfortunately, could not be continued. The following results may prove to be of some interest in that direction:

*Experiment 15. Kid 112 B* (*newly born, weight 1,350 gms.*).—A twin, which was not allowed to suckle mother, was removed immediately after birth and a blood sample was drawn. An immunized goat (15) was bled at once, the blood defibrinated and centrifuged to remove all corpuscles. About 30 c.c. of this supernatant serum was fed to the kid and no other feeding was made until the next day, when it was given cow's milk, which was continued from that time on. Unfortunately on testing this serum it was found to be of much lower value than suspected; test showed that 0.02 c.c. produced, however, a well marked hemolysis when tested by the regular technic, with the exception that 2.0 c.c. of 5 per cent instead of 7.5 per cent sheep blood suspension was used.

Samples of blood were taken upon four consecutive days after the feeding of the immune serum. When these samples (including the sample taken from the kid before feeding) were tested, in the manner just mentioned, none showed hemolysis in amounts of 0.15–0.20 c.c. If one-third of the antibodies were absorbed (unchanged) one would expect the test to give at least a slightly positive reaction in the volume of kid's serum which was tested. It follows that the absorption of unchanged antibodies from the digestive tract in this animal was not pronounced.

*Experiment 16. Kid 97 B* (*newly born, weight 2,535 gms.*).—Immediately after birth this kid was removed without being permitted to suckle. A blood sample was taken, then it was fed by pipette 15 c.c. of specific immune serum (goat 15) which had been kept under good condition, in freezing ice-box, for almost four months. This hemolytic serum, tested shortly after the feeding experiment, was of such potency that 0.002 c.c. almost completely hemolysed 3 c.c. of 5 per cent sheep blood suspension (0.10 c.c. guinea-pig serum for complement), while 0.001 c.c. gave a definite hemolysis. About one hour after this feeding the kid was given 150 c.c. of cow's milk. No further feeding was made until the next morning, when it was put upon cow's milk regularly. Blood samples were drawn daily at first, then at longer intervals, covering in all 15 days' observation. The tests were carried out upon these samples by the same technic (amount of blood samples, complement, and volume fluid) employed in the preceding experiment (Kid 112 B). It was found that the sample taken before the feeding of hemolytic serum was negative in 0.10 c.c.; the day following the immune serum feeding, 0.10 c.c. serum showed a well marked action; the second day after, the same amount of serum showed less hemolytic action; the hemolytic power gradually diminishing until, on the 13th and 15th days after the serum feeding, no action was shown by 0.10 c.c. This experiment showed that considerable absorption (approximately

10 per cent) of the unchanged hemolytic serum from the digestive tract took place, and appeared in the general circulation of the young animal. Recognizing the fact that no definite quantitative conclusions can be drawn from as few as two experiments, no more can be said than that these experiments suggest that antibody absorption while taking place is relatively meager when hemolytic sera are fed to newly born kids.

5. *General discussion of results.*—Before attempting to draw any definite conclusions from the above experimental data, it may be well to consider more fully some of the findings, and the related questions. In this brief discussion, not only our own results, but the correlated results of other workers, will be considered. The experiments clearly demonstrate that goats, when highly immunized against sheep erythrocytes during the latter part of gestation, transmit the specific antibodies to their sucklings. Apparently the placenta plays a very small part in this transmission, but the principal rôle is played by the antibodies which are present in the colostrum-milk and are readily absorbed from the digestive tract when taken by the newly born offspring. In those cases where the young born from immune mothers were prevented from taking the colostrum-milk until after a blood sample had been drawn, tests failed to show the presence of hemolysins in the kid's blood serum in sufficient amount to be appreciable by the method used. So we must conclude that the fetus *in utero* acquires from the actively immunized mother little if any hemolytic immune-body. We must therefore direct our attention particularly to the antibody bearing colostrum-milk, and the gastro-intestinal absorption of antibodies by the young.

Observations upon the colostrum, taken at time of parturition, and before the young were permitted to suckle, proved to be very interesting. When compared with the same animal's blood serum, taken at the same time and tested in parallel, it was sometimes found to be considerably higher in immune-body value, in some instances reaching a value two to three times greater than the blood serum. The published data of other workers would indicate that this occurs not infrequently where animals are immunized, some time before birth of young, against various antigens (Stäubli,[32, 80] Wegelius[24]). But particular attention has not been given to its importance. The question arises, Is the high hemolytic value of the colostrum in these cases due to (1) accumulated specific antibodies formed primarily in the mammary gland, or (2) a storage of antibody removed from the blood stream, or (3) are these antibodies present only in a concentration equivalent to that of the other body fluids but are augmented in action by other substances present in the colostrum, e.g., substances analogous to the "bovine colloid" of Bordet and Gay[72]?

As yet we know little concerning which tissues or organs form the hemolysins. The theory has been advanced, with some experimental support, that the blood-forming organs are the principal factors in the production of antibodies (Hektoen[73]). According to Hektoen,[69, 74] the blood itself, and probably the subcutaneous tissues at site of injection of blood corpuscles, do not fix the antigen and produce hemolysins to any appreciable extent. If this view concerning the formation of antibodies is accepted, little reason exists to suppose that the hemolysins are being formed to any extent in the mammary gland. No experimental proof has been brought forward showing that the cells of secreting glands produce antibodies. So, on the whole, little evidence exists which would support the first possibility.

Probably the second possibility mentioned is the true explanation of what actually occurs. In the absence of any direct experimental work on this particular question, certain facts may be considered, and a hypothesis deducted which will support the view. The quantitative relationship between the protein constituents of normal colostrum, and normal milk, in conjunction with known facts, must be considered. In the absence of full chemical data upon goat's colostrum and milk, the data upon analogous products from the cow must be used. According to König's[75] tables, the composition of goat's and cow's milk is very similar. Hoppe-Seyler-Thierfelder[76] states that colostrum is probably somewhat poorer in casein than milk, but very much richer in albumin and globulin. König's tables show practically the same general results with the exception of the casein, which he gives in a higher percentage for the average colostrum (4 per cent) than in the milk (3 per cent); he states as the average for coagulable protein (albumin and globulin) in colostrum, 13.6 per cent—for milk (albumin)

0.53 per cent. The colostrum contains an especially high content of globulin (Eichelberg[77]), while the milk contains only traces of this protein. At once we are struck by the great extremes of protein content existing between the colostrum and the milk from the cow. But it is also noticeable that cow's colostrum and milk do not show this great quantitative variation between their casein contents. Some observers report that the average casein content is about the same in colostrum and later milk. Casein is considered to be a nucleo-albumin, which is elaborated by the glandular epithelium and according to Mandel[78] probably originates in the gland cells from a breaking down of the nucleo-protein. So the casein content of the colostrum or milk may be used as an index of the secretory activity of the gland. The lacto-albumin which is present in the milk is also probably elaborated in the gland cells, and, with the casein, constitutes the normal milk proteins. The trace of lacto-globulin present in later milk has the properties of the sero-globulin, and probably is simply excreted by the gland cells by removal from the blood stream in an unchanged condition. In support of this supposition the work of Bauer[79] may be quoted. He prepared anti-sera by the injections of serum, colostrum, and milk. By a refined complement fixation test he was able clearly to differentiate blood serum and milk from the same animal. But when the anti-serum from cow colostrum was used, he found on testing that the highest specificity was shown against the same colostrum $\left(\frac{1}{100,000}\right)$ but also he had a fixation with cow's milk $\left(\frac{1}{10,000}\right)$ and ox serum $\left(\frac{1}{10,000}\right)$. These results showed that in colostrum there is present, not only the special milk proteins, but also those peculiar to the same animal's blood serum. His experiments would indicate also that the milk proteins were a specialized product of the milk glands. From both the purely chemical and the biologial reaction standpoint, it would appear that the proteins of colostrum and milk are somewhat different in qualitative character and in origin. The colostrum contains, in addition to those proteins of the normal milk, blood serum proteins, especially globulin, in considerable quantity.

It is a well known fact that during the active immunization of an animal its blood serum shows a decided increase in its protein content.* Also it is equally well known that antibodies themselves, if not protein in character, are closely associated with the proteins—in particular the serum globulins. Since we have seen that globulins are present in considerable quantity in colostrum, and that the biological test showed that colostrum contained serum protein while milk did not, we can assume that this is a seroglobulin, as only a trace of globulin is found in normal milk. The high concentration of antibodies in the colostrum might be explained by the excretion of serum proteins (globulin, etc.) by the glandular ephithelium, and incidentally the specific antibodies which are probably closely associated. The antibody value, if the hypothesis is true, would parallel the protein concentration in the colostrum.

Stäubli [32, 80] made similar observations upon typhoid agglutinin-colostrum and blood serum. He found that the colostrum from a clinical case, as well as animal experiments, showed much higher agglutinin content than the blood serum at the same time. He thought that perhaps the milk glands took up the antibody from the blood and returned the water, thus causing a concentration; and also that a certain parallelism existed between the coagulable proteins (lacto-albumin, lacto-globulin) and the agglutinins. He considered the gland cells as playing an important *active* rôle in the formation of agglutinins, and that the high value of colostrum was influenced by the destruction of those cells.

The possibility that substances may be present in colostrum, which might augment the action of the hemolysins, offers some points of theoretical interest. But the supposition is hardly tenable as an explanation, since the dilution of the colostrum was so great in many instances that a "colloidal action" would be negligible. Other chemical substances present would also suffer in the same way. These substances would also appear in the normal colostrum drawn from the animal before immunization, but in no case did we ever get a hemolysis from such a colostrum, in the

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*Ledingham (*Jour. Hyg.*, 1907, 7, p. 65) reported quantitative protein changes in serum of a goat during an immunization against diphtheria toxin. The total protein in the normal serum before injection of toxin was 5.867 per cent; the maximum reached during the course of immunization was 8.555 per cent, then gradually diminished. Gibson and Banzhaf (*Jour. Exper. Med.*, 1910, 12, p. 411) never found the total proteins to rise above 10.52 per cent during the active immunization of horses (11 animals) against diphtheria or tetanus toxins.

presence of rabbits or guinea-pig complement, even when used in large quantities.

Before leaving this side of the question we wish to say that it is highly desirable that further work should be carried out along these particular lines.

The gastro-intestinal absorption of unchanged antibodies from immune serum has received attention ever since the introduction of serum therapy. Escherich[81] attempted to passively immunize children by giving antidiphtheritic serum *per os*. He reported that infants of the first months showed a slight rise of antitoxin in their blood, but in older children he met with no success. Laboratory animals failed to absorb appreciable amounts of antitoxin, even when fed in relatively large quantities (Carrière[82]). The work of the v. Behring school (Behring[17], Ransom[57], Roemer[83]) confirmed the findings that adult animals under normal conditions do not absorb antitoxic serum (heterologous) in an unchanged condition, but the newly born animals do so readily from the mother's milk (homologous). They attempted to explain this on the basis of the differences between the anatomical structure of the gastro-intestinal mucosa in the newly born and the grown (Disse[84]), but these claims have not been verified. At the present time certain other factors are considered very important in this process. Salge[85] fed antidiphtheritic serum to infants, but states that no antitoxin passed over to the blood. He observed that infants getting antitoxin through the milk of the nurse readily absorbed it. He considered this difference to be due to homologous antibodies in the milk which were easily absorbed, while the heterologous antidiphtheritic serum was not assimilated in an unaltered state. Salge,[86] in order to test this point, fed infants for several weeks with goat milk (heterologous) drawn from immunized animals and showing considerable antitoxic value, but was unable to demonstrate the presence of antibodies in the blood of the infants. The work of Bertarelli[87] and others would tend to support the same view, although this point is not fully settled.

In my experiments it was seen that newly born kids readily absorbed hemolysins when taking the mother's colostrum-milk containing these bodies. Also in one case, in which the homologous hemolytic serum (highly potent) was fed, it was absorbed in considerable amounts but probably not so readily as the colostrum-milk hemolysins. Uffenheimer[88] in his extensive work upon gastro-intestinal absorption (bacteria, proteins, antibodies) touched upon the absorption of hemolysins. He fed guinea-pigs, newly born or during the first days of life, considerable amounts of hemolytic serum derived from rabbits immunized against guinea-pig blood cells. He concluded that none of the hemolysins was absorbed, since the young animals gave no evidence of intoxication (hemoglobulinuria, decrease in number of blood cells, etc.). Much smaller doses of the same serum when injected into controls caused their death.

The reports of several investigators (Ganghofner u. Langer,[89] Uffenheimer,[88] Roemer u. Much[90]), upon the question of the gastro-intestinal absorption of unchanged proteins, etc., by young animals, call attention to various factors which influence the absorption, such as age, overfeeding of proteins, the nature and source of the proteins, digestive powers, irritation or lesions of the intestinal mucosa, species of animal, individual variation, etc. In considering the possibility of immunizing the young animal through the digestive tract, the above factors must be kept in mind.

The failure to transmit immune bodies to the suckling when the mother is immunized after its birth, in all probability, is chiefly due to two reasons: first, the milk immune bodies do not appear in the milk at any time in very great quantities; while secondly, the time of their appearance is past the critical age point where the young are able to absorb the hemolysin (unchanged) to any extent.

When immunization against sheep corpuscles was carried out in goats during the period of lactation, the antibody content of the milk when compared with that of the serum was never greater than 1:45–50. Similar observations upon goats immunized against diphtheria and tetanus showed that the milk contained relatively greater amounts of antitoxin when compared with the serum, than shown in the above ratio for hemolysins (Ehrlich u. Wassermann[91]).

All observers are agreed in the conclusion that the newly born animal (or infant) absorbs from the digestive tract unaltered antibodies most readily when given immediately after birth. The most effective means of supplying the antibodies to the young is through the mother's colostrum-milk (homologous); probably next in importance is the homologous serum, and finally the antibody milk or serum from some other species.

It follows from our observations that of all factors the colostrum plays the most important rôle in transmitting antibodies from the actively immunized mother to the young. Even under normal conditions, it is reasonable to suppose that protective substances are concentrated in the colostrum from the mother's body fluids and may be in part absorbed by the infant after the first feedings. Consequently it seems of highest importance that the newly born child should, during the first days of life, receive the colostrum-milk, unless it is contra-indicated for some special reason. Perhaps the natural resistance which many infants show against many infectious diseases may be partially explained on the basis that the child is passively immunized by anti-substances excreted through the immune mother's colostrum and milk, which are absorbed by nurslings during the early days of life. Such a view gains strength from the observations of Moro,[92] who found that the blood serum from breast-fed infants showed greater bactericidal and hemolytic powers than that from infants who were artificially fed upon cow's milk. The practical application, of adding to the mother's immunity by vaccines during pregnancy, would undoubtedly be attended with risk of disturbing the natural course of pregnancy, and should not be entered into unless further investigation demonstrates its safety.

6. *Summary.*—To summarize briefly the principal results of the foregoing experiments, it was found that:

1. Goats actively immunized against sheep blood corpuscles during gestation passively transmitted the specific hemolysin to their young.

2. The colostrum was the chief agent in bringing about the passive immunization of the suckling. Sucklings which got the colostrum and first milk rapidly acquired a relatively high antibody content in their blood, which was well retained.

3. When the immunization was done during the period of gestation the colostrum contained a high content of specific

hemolysin, often much higher than the adult's serum, at time of parturition.

4. The hemolytic antibodies rapidly disappeared from the milk after the mother had been suckled by the young.

5. The blood taken from the newly born before they were permitted the antibody colostrum showed no appreciable amount of hemolysin by the test used. The placenta played a minor rôle in the passage of hemolysins to young before birth, practically negligible in most cases.

6. Mother goats, actively immunized against sheep-blood corpuscles immediately after birth of their young, failed to transmit any demonstrable immunity to their suckling young.

7. The milk, in some cases, contained no demonstrable hemolysins, but in others showed fairly large amounts.

8. Apparently a very high degree of immunity is necessary before appreciable amounts of antibodies are excreted through the milk.

9. Older sucklings apparently did not absorb the antibodies in an unchanged condition.

10. The young animals (kids) did not respond to any extent in production of hemolysins following subcutaneous injections of foreign blood cells (sheep).

In conclusion, I wish to express my indebtedness to Dr. William H. Park, Director of Laboratories, Department of Health, for many helpful suggestions in connection with the above work. I am also indebted to Drs. Paul Bartholow, and Dr. Maria Grund, for valuable assistance.

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